Remarks

Upon entry of the foregoing amendments, claims 68-120 are pending in the application, with the independent claim being claim 68. Claims 7-16 and 43-67 have been canceled without prejudice to or disclaimer of the subject matter therein. New claims 68-120 have been added. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Support for the Amendments

The Examiner has required Applicant to submit substitute pages 10-11, 45, 54, 64, 66, 76-77, 79, 81, and 83 under 37 C.F.R. § 1.125(a), alleging an excessive number of amendments to those pages. Due to the vagaries of word processing and printer formats, and for the sake of clarity, Applicant has opted to submit herewith a complete substitute specification under 37 C.F.R. § 1.125(b). Except for miscellaneous corrections of typographical errors, and corrections as to form of the application, all as noted in the marked up copy of the substitute specification, the changes to the specification include only those made in the Third Preliminary Amendment filed on May 10, 1999. The substitute specification has been amended relative to the specification of record to provide complete cross-references between the specification and the Sequence Listing, to remove the Table of Contents, to remove the numbering from headings and subheadings, to renumber the Examples to conform with standard practice, and to correct inadvertent typographical errors. Pursuant to 37 C.F.R. § 1.125(b)(1), the undersigned hereby states that the substitute specification includes no new matter. As required by 37 C.F.R. § 1.125(b)(2),

Applicant also submits herewith a marked up copy of the substitute specification showing where specific amendments have been made relative to the specification of record. The marked up copy of the substitute specification was prepared using the CompareRite™ Program. Deletions appear as Strikethrough text (in red) surrounded by brackets, and additions appear as double-underlined text. Although no changes were made to any of the tables throughout the specification, the differences in table formatting between the original specification (prepared in a different word processing program) and the substitute specification appear as changes in the marked up version.

Claims 68-120 have been added to more particularly point out and distinctly claim the subject matter Applicant regards as the invention. Support for the new claims can be found throughout the substitute specification and original claims. Specifically, support for claims 68 and 117-120 may be found, *inter alia*, in claims 1 and 7 as filed, and in the substitute specification at page 8, lines 1-2, 7-11, 14-17, and 25-30; page 13, line 27- page 14 line 2; page 15, line 15-19; page 21, line 1-17; page 22, line 22-25, and page 50, line 21-26. Support for claim 69 may be found, *inter alia*, in the substitute specification at page 13, line 27-page 14, line 2, and page 26, lines 11-14. Support for claim 70 may be found, *inter alia*, in the substitute specification at page 50, line 27 - page 51, line 12. Support for claim 71 may be found, *inter alia*, in the substitute specification at page 30, line 21 - page 31, line 12. Support for claims 72, 74, and 78 may be found, *inter alia*, in claims 4 and 14 as filed, and in the substitute specification at page 6, lines 11-12, page 19, lines 26-30, and page 20, lines 25-26. Support for claims 73 and 75 may be found, *inter alia*, in the substitute specification at page 46, lines 8-13. Support for claims 76 and 102 may be found, *inter alia*, in the substitute specification at page 6, lines 11-15. Support for claims 77 and 103 may be found, *inter alia* in claim 16 as filed, and in the substitute specification at page 13, lines 14-16. Support for claim 79 may be found, *inter alia*, in the substitute specification at

page 25, lines 3-5 and page 42, lines 19-23. Support for claim 80 may be found, *inter alia*, in the substitute specification at page 6, lines 16-20, page 9, lines 9-11, and in Figure 1. Support for claim 81 may be found, *inter alia*, in the substitute specification at page 72, line 2, and page 78, lines 14-15 and 19-22. Support for claims 82-85 may be found, *inter alia*, in the substitute specification at page 78, lines 19-22, and in Figure 1. Support for claims 86 and 87 may be found, *inter alia*, in the substitute specification at page 42, lines 21-23, and page 43, lines 8-10 and 14-17. Support for claim 88 may be found, *inter alia*, in the substitute specification in the legend to Figure 2, in Figure 2, and in SEQ ID NO:6. Support for claim 89 may be found, *inter alia*, in the substitute specification at page 9, lines 16-21. Support for claims 90 and 91 may be found, *inter alia*, in the substitute specification at page 9, lines 18-21, in SEQ ID NOs: 7, 8, and 9, and in Figure 2. Support for claim 92 may be found, *inter alia*, in the substitute specification at page 1, lines 14-17, and page 14, lines 7-8. Support for claim 93 may be found, *inter alia*, in the substitute specification at page 4, lines 3-5, page 8, lines 9-11, and page 13, lines 6-8. Support for claims 94-100 and 104-115 may be found, *inter alia*, in the substitute specification at in Example 1, pages 37-46. Support for claim 101 may be found, *inter alia*, in the substitute specification at page 6, liens 11-15. Support for claim 116, may be found, *inter alia*, in the substitute specification at page 9, liens 13-16, and in Figure 1.

Thus, the added claims do not incorporate new matter.

Accordingly, entry of the foregoing amendments is respectfully requested.

The Restriction Requirement

Applicant affirms the election of the claims of Group VI (not Group IV as stated in Paper No. 18), directed to a method for selecting recombinants encoding a tumor target epitope, and accordingly requests the cancellation of claims 7-16 and 62-66.

Applicant retains the right to petition from the restriction requirement under 37 C.F.R. § 1.144.

Although Applicant has further made a species election of a method of selecting for test recombinants comprising the species of a vaccinia virus vector and trimolecular recombination, Applicant asserts the right to claim additional species in the event that a generic claim thereto is found to be allowable in accordance with 37 C.F.R. § 1.141(a).

Rejection under 35 U.S.C. § 112, Second Paragraph

(a) The Examiner has rejected claims 43-37, 49-58, 60-61, and 67 under 35 U.S.C. § 112, second paragraph, arguing that the term "recombinants" (*e.g.*, in claims 43, 46-47, and 49-51) is indefinite because it is not defined in the specification. *See* Paper No. 18 at page 3.

While not acquiescing to the Examiner's rejection, Applicant has canceled claims 43-67, and has added claims 68-120. The added claims are drawn to a method for selecting a nucleic acid molecule encoding a target epitope of specific T-lymphocytes, which method comprises screening a library of heterologous nucleic acid molecules carried in a vector capable of facilitating expression of the target epitope in a host cell.

(b) The Examiner has rejected claims 43-47, 49-58, 60-61, and 67 under 35 U.S.C. § 112, second paragraph, alleging that the phrase "a first population," *e.g.*, in claim 43, has not

been defined and implies the existence of an allegedly undefined second population. *See* Paper No. 18 at page 3.

While not acquiescing to the Examiner's rejection, Applicant has canceled claim 43-47, 49-58, 60-61, and 67 and have added claims 68-120. Applicant notes that claim 43 referred to "a first population of adherent cells," and claim 45 referred to "a second population of adherent cells," the distinction being made to show that the method of claim 43 was further limited in claim 45 by requiring the process to be repeated in a second group of adherent cells to further select the desired recombinant molecules. Added claim 70 further limits added claim 68 in the same way, however, applicant has, in both claims, referred to "host cells" generically. In both claims, any host cell is intended, as long as it meets the criteria of the claim, *i.e.*, a cell that will support expression of the target epitope, and a cell that expresses a defined MHC molecule.

(c) The Examiner has rejected claim 60 under 35 U.S.C. § 112, second paragraph, stating that the term "trimolecular recombination" is indefinite because it is not defined in the specification. *See* Paper No. 18 at page 3.

While not acquiescing to the Examiner's rejection, Applicant has canceled claim 60, and has added claims 94 and 98, which particularly point out the aspects of trimolecular recombination as defined in the specification, *e.g.*, in Example 1, pages 37 to 46 of the substitute specification. In particular, Applicant notes that "trimolecular recombination," as defined in the specification, denotes *in vivo* homologous recombination between (1) "said first viral fragment", (2) "said second viral fragment," and (3) "said transfer plasmids." See, *e.g.*, the substitute specification at page 41, lines 6-10, and page 46, lines 9-13.

(d) The Examiner has rejected claims 43-47, 49-58, 60-61, and 67 under 35 U.S.C. § 112, second paragraph, alleging the omission of essential elements amounting to a gap between the elements. *See* Paper No. 18 at page 3.

While not acquiescing to the Examiner's rejection, Applicant has canceled claims 43-47, 49-58, 60-61, and 67. Added claim 68 recites clear relationships between the elements. For example, it is pointed out that a library of heterologous nucleic acid molecules, at least one of which encodes a target epitope, is expressed in a population of host cells, and that a host cell expressing the target epitope undergoes a lytic event upon contact with an specific cytotoxic T-lymphocyte. Subsequently, the host cells which have undergone a lytic event are recovered.

In view of these remarks, Applicant respectfully requests that the Examiner reconsider and withdraw all rejections under 35 U.S.C. § 112, second paragraph, as applied to the pending claims.

Rejections under 35 U.S.C. § 103

(a) The Examiner has rejected claims 43-44, 46-47, 49-52, 56-67, 60-61, and 67 under 35 U.S.C. § 103(a) as allegedly being rendered obvious by U.S. Patent No. 5,843,648 (the '648 patent) in view of U.S. Patent No. 5,866,383 (the '383 patent) and Scheiflinger *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*:9977-9981 (1992); and claim 45, further in view of Sambrook, *et al.*, (1989). The Examiner alleges, *inter alia*, that the '648 patent teaches "a method of *selecting* recombinants comprising nonviral DNA constructed in a vaccinia (poxvirus) viral vector . . . encoding a target epitope comprising contacting a population of adherent cells containing test recombinants . . . with cytotoxic T lymphocytes . . . (*which lyse*

the target cells) and *collecting cells which react with the CTL* and recovering recombinant DNA." *See* Paper No. 18 at page 4 (emphasis added). Claims 43-47, 49-52, 56-67, 60-61, and 67 have been canceled. With respect to the currently pending claims, Applicant respectfully traverses.

In order to establish a *prima facie* case of obviousness, the Examiner must establish that the cited art *teaches or suggests all elements of the claimed invention. See In re Deminski*, 230 U.S.P.Q. 313, 315 (Fed. Cir. 1986). The method of claim 68 requires, *inter alia*, the *selection* of a target epitope of specific T-lymphocytes from *an expression library* harbored in host cells which express a specific MHC molecule. The method specifies that selection is based on the fact that a host cell expressing the target epitope *undergoes a lytic event* upon interaction with a specific cytotoxic T-lymphocyte. *Recovery* of those host cells harboring the desired recombinant vector is made by collecting the actual host cells which have undergone a lytic event. From these recovered cells, the nucleic acid molecule encoding the desired target epitope is isolated.

The '648 patent does not teach or suggest all of the elements of claim 68. The '648 patent lists expression in vaccinia virus vectors as one of a long list of eukaryotic expression systems in which to express the claimed P15 and tyrosinase melanoma antigens. *See* '648 Patent, col. 7, line 47. The context of this reference, however, is directed to expression of a *previously isolated* cDNA encoding a p15 tumor antigen. The '648 patent does not teach or suggest the use of a vaccinia virus vector for construction of an expression library. Expression of a previously-isolated cDNA in a vaccinia vector, as taught in the '648 patent, is distinct

from the cDNA library of claim 68, *i.e.*, a population of host cells including many diverse cDNA clones, constructed in a vaccinia virus vector by trimolecular recombination.[1]

More importantly, the '648 patent does not teach or suggest *selection* of a recombinant host cell encoding a target epitope, as required by claim 68. In particular, it does not teach that the host cell undergoes a lytic event upon interaction with a specific cytotoxic T-lymphocyte, and it does not teach *recovery of the specific host cells which react with the CTL*, as alleged by the Examiner.[2] The '648 patent teaches isolation of the p15 antigen through use of a *screening* assay, not a selection. In the method taught in the '648 patent, cloned plasmid DNAs were first isolated from pools of 50-100 bacterial transformants, and were used to transiently transfect eukaryotic cells. *See* col. 23, lines 2-21. These pools were then screened for the ability to stimulate the release of GM-CSF from a specific CTL clone. Host cells carrying the desired plasmids were not recovered from the transiently transfected eukaryotic cells, rather, once a positively reacting pool was identified, individual plasmids were isolated from the earlier-preserved bacterial transformants, used to transfect eukaryotic cells, and the screening process was repeated until a unique cDNA clone was identified. In the screening assay described in the '648 patent, 176 pools were separately assayed to identify the pool containing a positive clone. *See* col. 25, lines 6-17. The *screening* assay of the '648

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[1] Applicant wishes to point out that even though the Examiner has required a species election and has thus limited the examination to the species of vaccinia virus recombinants constructed through trimolecular recombination, claim 68 remains *generic* with respect to the vector in which the library of heterologous nucleic acid molecules is constructed.

[2]The only references to CTL-mediated target cell lysis in the '648 patent refer to the well-known techniques of CTL-mediated lysis of certain melanoma target cells isolated from patients, and the use of previously isolated and characterized peptide epitopes to sensitize cultured cells to CTL-mediated lysis. *See, e.g.*, col. 24, lines 14-25, and col 30, lines 65-67. The '648 patent does not teach the use of CTL-mediated cell lysis as a method for selecting a nucleic acid molecule encoding a CTL-reactive epitope from a library.

patent differs from the *selection* assay of claim 68 of the captioned application, in that the host cells do not undergo a lytic event, and host cells are not recovered, or selected, from the assay based on their CTL reactivity. Applicant's selection method, as typified in claim 68, is far more efficient than the screening process described in the '648 patent, since it is not necessary to separately assay the many negative pools and clones. In other words, the claimed method may be carried out using only one "pool," which contains all the clones, both positive and negative.

The Examiner argues in favor of *prima facie* obviousness based on the premise that "collection of said lysed cells or the continued growth of unlysed cells of the same clone, result in the same outcome . . . ." The Examiner's argument is misguided, because Applicant is not claiming an "outcome." Rather, Applicant is claiming a *method* of selecting a nucleic acid molecule encoding CTL-reactive target epitope represented in an expression library, comprising collecting the *actual* host cell expressing the epitope, through its reaction with CTL. The '648 patent does not teach, or even suggest collecting the actual host cells which react with CTL. Accordingly, the '648 patent does not teach or suggest all the elements of claim 68. Since each of claims 69-120 depend from, and therefore include all the elements of claim 68, the '648 patent does teach or suggest all the elements of these claims either.

Neither the '383 patent, Scheiflinger *et al.*, nor Sambrook *et al.* cure the deficiencies of the '648 patent. The '383 patent teaches the construction of recombinant vaccinia virus by *in*

vitro ligation. Scheiflinger *et al.* teaches the use of a fowlpox helper virus to facilitate the packaging of vaccinia virus DNA.[3] Sambrook *et al.* teaches the use of multiple rounds of *screening*. None of these references teach or suggest the *selection* of a CTL-reactive host cell by virtue of a lytic event, nor recovery of those host cells which have undergone a lytic event. Since the '648 patent, the '383 patent, Scheiflinger *et al.*, and Sambrook *et al.* cannot be combined in a way that teaches or suggests all of the elements of claim 68, withdrawal of this rejection is respectfully requested.

(b) The Examiner has rejected claims 43-44, 46-47, 49-52, 56-67, 60-61, and 67 under 35 U.S.C. § 103(a) as allegedly being rendered obvious by U.S. Patent No. 5,874,560 (the '560 patent) in view of U.S. Patent No. 5,866,383 (the '383 patent) and Scheiflinger *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*:9977-9981 (1992); and claim 45, further in view of Sambrook, *et al.*, (1989). Similar to the '648 patent, the Examiner alleges that the '560 patent teaches "a method of *selecting* recombinants comprising nonviral DNA constructed in a vaccinia (poxvirus) viral vector . . . encoding a target epitope comprising contacting a population of adherent cells containing test recombinants . . . with cytotoxic T lymphocytes . . . (*which lyse the target cells*) and *collecting cells which react with the CTL* and recovering recombinant DNA." *See* Paper No. 18 at page 5 (emphasis added). Claims 43-47, 49-52, 56-

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[3] Although it is not directly relevant to Applicant's traversal of the present rejection (*see* note 1, *supra*), Applicant notes that Scheiflinger *et al.* does *not* teach the method of trimolecular recombination as defined in the present application, and claimed in added claims 94 and 98. In fact, the major point of Scheiflinger *et al.*, as well as the '383 patent, is that *in vivo* homologous recombination is not required. Instead, heterologous DNA is inserted into the genome through *in vitro* ligation followed by *in vivo* packaging through use of a helper fowlpox virus. It is inaccurate to describe this as "trimolecular recombination" as the Examiner has done, since the method is independent of homologous recombination of any kind, either *in vitro* or *in vivo*.

67, 60-61, and 67 have been canceled. With respect to the currently pending claims, Applicant respectfully traverses.

The '648 patent does not teach or suggest all of the elements of claim 68. The '560 patent lists expression in vaccinia virus vectors as one of a long list of eukaryotic expression systems in which to express the claimed MART-1 melanoma antigen. *See* '560 Patent, col. 7, line 38. The context of this reference, however, is directed to expression of a *previously isolated* cDNA encoding a MART-1 tumor antigen. The '560 patent does not teach or suggest the use of a vaccinia virus vector for construction of an expression library. Expression of a previously-isolated cDNA in a vaccinia vector, as taught in the '560 patent, is distinct from the cDNA library of claim 68, *i.e.*, a population of host cells including many diverse cDNA clones, constructed in a vaccinia virus vector by trimolecular recombination.[4]

More importantly, the '560 patent does not teach or suggest *selection* of a recombinant host cell encoding a target epitope, as required by claim 68. In particular, it does not teach that the host cell undergoes a lytic event upon interaction with a specific cytotoxic T-lymphocyte, and it does not teach *recovery of the specific host cells which react with the CTL*, as alleged by the Examiner. [5] The '560 patent teaches isolation of the MART-1 antigen through use of a *screening* assay, not a *selection*. In the method taught by the '560 patent,

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[4] *See* Note 1, *supra*.

[5] The only references to CTL-mediated target cell lysis in the '560 patent refer to the well-known techniques of CTL-mediated lysis of certain melanoma target cells isolated from patients (chromium release assays), evaluation of individual cDNA-transfected cell lines, cell lysis in a patient when a characterized clone is used as a vaccine, and the use of previously isolated and characterized peptide epitopes to sensitize cultured cells to CTL-mediated lysis. *See, e.g.*, col. 16, lines 1-4, col. 22, lines 41-42, col. 24, lines 21-27, and col. 32, lines 16-23. The '560 patent does not teach CTL-mediated cell lysis as a method for selecting a nucleic acid molecule encoding a CTL-reactive epitope from a library.

individual plasmid cDNA clones were stably transfected into mammalian cells, and these stably transfected cell lines were separately expanded and preserved. Replicate cultures of the various transfected cell lines were contacted with CTL, and the supernatants were collected and assayed for the release of IFN-γ. *See* col. 24, line 66- col. 25, line 11. Host cells carrying a plasmid expressing the desired epitope were *not* recovered directly from this assay, rather, once a positively reacting cell line was identified, individual inserts were isolated from the previously preserved replicate cell lines by PCR, followed by subcloning into a eukaryotic expression vector. In the screening assay described in the '560 patent, out of 13,400 cell lines individually isolated and individually screened, 15 positive clones were detected. *See* col. 26, lines 23-67. The *screening* assay of the '560 patent differs from the *selection* assay of claim 68 of the captioned application, in that each stably-transfected host cell line harboring an isolated, cDNA clone must be individually expanded, the assay does not require the host cells to undergo a lytic event, and host cells are not recovered, or selected, from the assay based on their CTL reactivity. Applicant's selection method, as typified in claim 68, is far more efficient than the screening process described in the '560 patent, since it is not necessary to individually isolate, expand, and assay the many negative clones.

As with the '648 patent, the Examiner argues in favor of *prima facie* obviousness based on the premise that "collection of said lysed cells or the continued growth of unlysed cells of the same clone, result in the same outcome . . . ." The Examiner's argument is again misguided, because Applicant is not claiming an "outcome." Rather, Applicant is claiming a *method* of selecting a CTL-reactive target epitope represented in an expression library, comprising collecting the actual host cell expressing the epitope, through its reaction with CTL. The '560 patent does not teach, or even suggest collecting the actual host cells which

react with CTL. Accordingly, the '560 patent does not teach or suggest all the elements of claim 68. Since each of claims 69-120 depend from, and therefore include all the elements of claim 68, the '560 patent does teach or suggest all the elements of these claims either.

Neither the '383 patent, Scheiflinger *et al.*, nor Sambrook *et al.* cure the deficiencies of the '560 patent. The '383 patent teaches the construction of recombinant vaccinia virus by *in vitro* ligation. Scheiflinger *et al.* teaches the use of a fowlpox helper virus to facilitate the packaging of vaccinia virus DNA.[6] Sambrook *et al.* teaches the use of multiple rounds of screening. None of these references teach or suggest the selection of a CTL-reactive host cell by virtue of a lytic event, nor recovery of those host cells which have undergone a lytic event. Since the '560 patent, the '383 patent, Scheiflinger *et al.*, and Sambrook *et al.* cannot be combined in a way that teaches or suggests all of the elements of claim 68, withdrawal of this rejection is respectfully requested.

Based on these remarks, Applicant respectfully requests that the rejections under 35 U.S.C. § 103, as applied to the pending claims, be withdrawn.

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[6] *See* Note 3, *supra*.

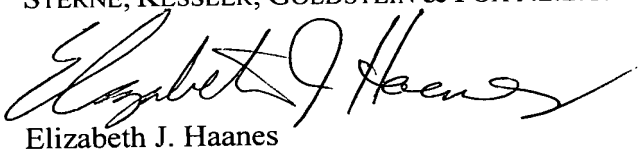
Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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